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THÈSE

de Doctorat d'Etat ès Sciences Naturelles

présentée

A L'UNIVERSITÉ DE PARIS VII

par

ÉLISABETH SWIERCZEWSKI

pour obtenir

le grade de Docteur ès Sciences

SUJET DE LA THÈSE

GLUTATHION-PEROXYDASE

1-Étude de son développement dans le foie et les erythrocytes durant normale et hypotrophique
2-Rôle dans la réactivation de la ribonucléase réduite par le GSH.

soutenue le 17^e Juin 1971 devant la Commission d'Examen

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Centre de Recherches Biologiques Néonatales, Unité INSERM, Hôpital Port-Royal, Paris
(Director: Prof. A. MINKOWSKI)

Glutathione Peroxidase in Rat Liver during Development

II. Changes in Glutathione Peroxidase during Post-Natal Development of Normal and Hypotrophic Rats

ANNMARIE DEMUS-OOLE and ELISABETH SWIERCZEWSKI

Introduction

In the preceding publication [1] we reported on developmental changes of glutathione peroxidase which occurred in rat liver. Very low values of activity were found in newborn rats at birth, as compared to four-month-old rats, in all fractions of liver tissue.

Changes in activity of many enzymes were described during mammalian development. For instance as shown by DAWKINS [2] and LANG [3], electron transport enzymes, among others, increase in rat liver three to sixfold during the post-natal period.

Some communications mentioned before have shown the existence of a possible relation between the electron transport system and GPX in the mitochondria [4].

Our purpose in this study was to follow the activity of GPX in the soluble fraction of rat liver during development from birth to adult age.

Experimental Procedure

Preparation of liver extracts, assay for measurement of GPX activity, were carried out as described previously [1].

The enzyme source for all experiments was the supernatant solution from a liver homogenate centrifuged at 100,000 $\times$ g for 20 min.

Animals. In these experiments we used liver of rats from the Sherman strain: newborn rats after 21.5 days of gestation, rats at 24, 48, 72 h, 8 days of life, rats during puberty (one-month-old) and adult male and female (four-month-old).

Hypotrophic rats at different stages of development were also studied. They were experimentally obtained by the method described below.

Production of hypotrophic rat fetuses by the method of WIGGLEWORTH [5]. Female rats of the Sherman strain were used. The uterine vessels were clamped near the lower end of one uterine horn on the 17th day of pregnancy. The other horn was left as a control. The

fetuses were born at term by normal delivery. The reduction of body weight of the hypotrophic fetuses was about 50% when compared to the control.

Results

GPX activity of soluble liver fraction in developing normal rat. The activity of GPX expressed as μ moles of NADPH per minute per gram liver wet weight is very low at birth and increases about 20 times from birth to 4 months of age (fig. 1).

The mean activities for each stage, expressed per gram liver or per mg protein in the assay are seen in table I. Each group studied includes ten animals. To be particularly noted is that at 48 h a significant decrease of activity appears, and the initial value found at birth is not reached again, till the 8th day (fig. 2).

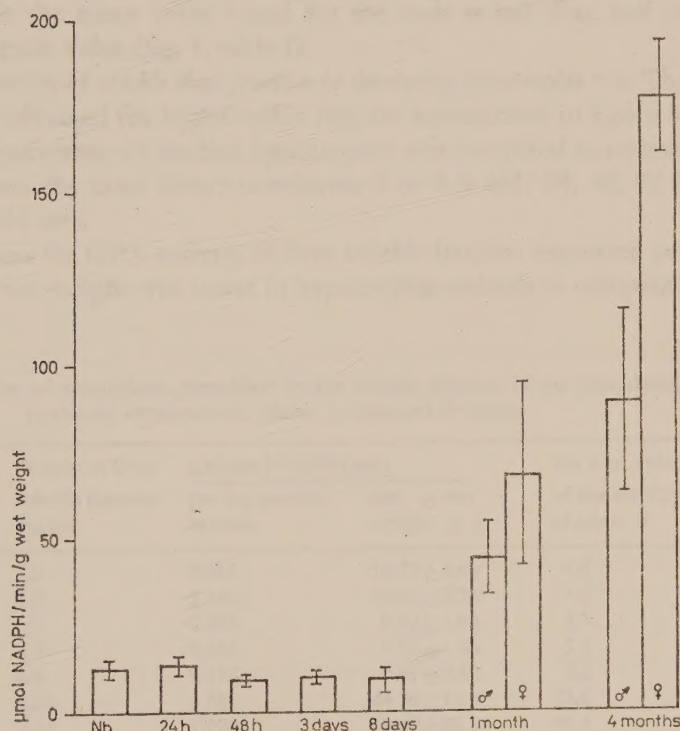


Fig. 1. Evolution of GPX activity during development. Each column represents the mean value $\pm$ standard deviation.

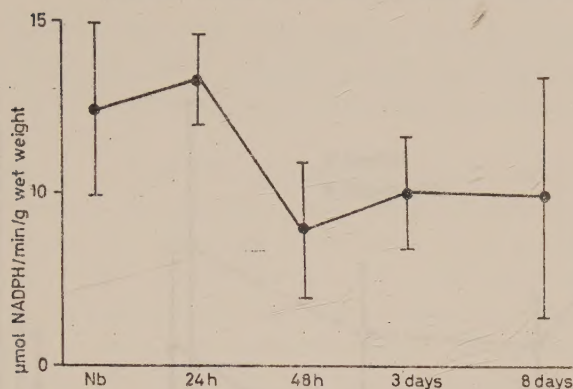


Fig. 2. Activity of GPX during the first days after birth. Mean values $\pm$ standard deviations.

Differences in activity linked to sex appear at one month of age, female manifesting considerably higher activities than the male. At four months the mean value found for the male is less than half of the adult female value (fig. 1, table I).

GPX activity of soluble liver fraction in developing hypotrophic rat. The first results obtained for hypotrophic rats are summarized in figure 3. In these experiments we studied hypotrophic rats compared to normal controls from the same litter: new-borns 3 to 6 h old; 24, 48, 72 h and 5-day-old rats.

In all cases the GPX activity of liver soluble fraction expressed per gram liver wet weight was lower in hypotrophic animals as compared

Table I. Activity of glutathione peroxidase in the soluble fraction of rat liver during post-natal development. Means $\pm$ standard deviation

Age	Protein in liver soluble fraction mg/ml	μ Moles NADPH/min		Per cent values of the activity of adult ♀
		per mg protein in assay	per .g wet weight $\pm$ s	
Newborns	7.8	0.153	12.42 $\pm$ 2.46	6.6
24 h	8.2	0.163	13.26 $\pm$ 2.34	7.1
48 h	9.7	0.098	8.92 $\pm$ 1.99	4.7
72 h	6.9	0.132	9.98 $\pm$ 1.56	5.3
8th day	6.9	0.132	9.87 $\pm$ 3.52	5.2
1 month ♂	6.25	0.681	44.50 $\pm$ 10.9	23.6
1 month ♀	6.25	1.178	68.60 $\pm$ 26.5	36.4
4 months ♂	6.25	2.395	89.90 $\pm$ 26.0	47.7
4 months ♀	6.25	2.944	188.40 $\pm$ 26.6	100.0

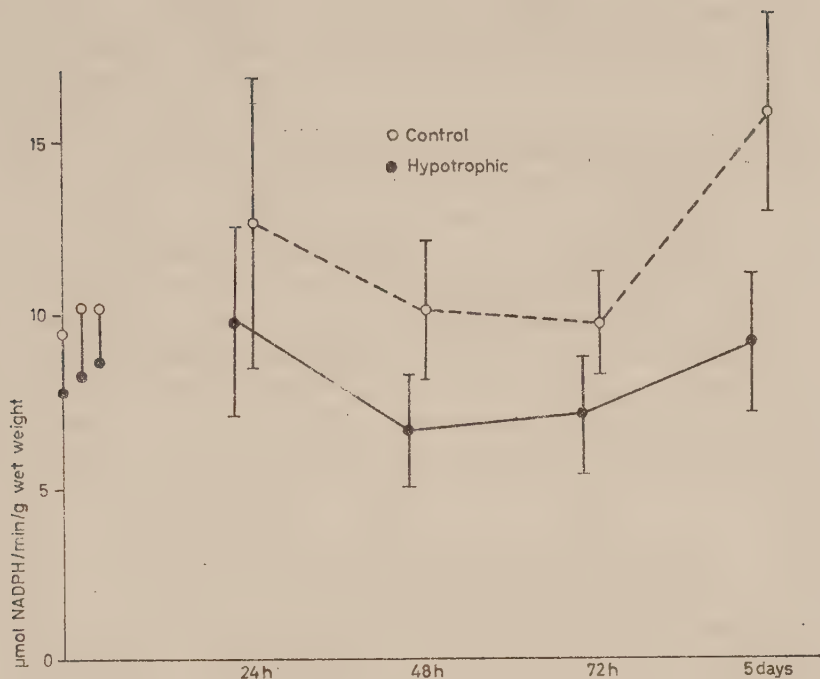


Fig. 3. Comparison between GPX activities of hypotrophic and normal rats from the same litter, during the first days of post-natal development. Mean values $\pm$ standard deviations. $n=7$.

to the control. The mean difference increases from about 23% at birth to 42% at the 5th day.

Further studies investigating the period of prepuberty to adulthood are at the moment being carried out. Results will be published in the near future.

Discussion

Studying the profile of the enzyme GPX during development, in the soluble fraction of post-natal rat liver, we could describe approximately three periods. A low activity after birth with a slight fall at 48 h; a first rise between 8 days and one month of age. During this period the evolution was similar in males and females. A sharp pubertal rise occurred in females from one to four months of age. A short communication [6] published at the end of our experiments reported changes in glutathione reductase and GPX activities in rat

liver, from foetal to adult life. The authors also described some variations of both enzymatic activities during the oestrous cycle. These results probably obtained with total liver homogenate, are in agreement with ours.

The pattern of soluble GPX during rat development is very similar to the pattern described by LANG [3] for the NADH cytochrome c reductase. In both cases adult values are not reached for several weeks after birth; NADH cytochrome c reductase constitutes an exception to the general pattern of development of the respiratory enzymes which present a very rapid increase in activity after birth.

As seen before, some relations were found between the electron transport system and the GPX function in the mitochondrial fraction. The developmental increase of GPX activity in the mitochondrial fraction may be correlated with enhanced oxidative processes in the liver mitochondria.

The exact role of GPX of the soluble fraction is rather obscure. O'BRIEN and LITTLE [7] described in supernatant fractions of liver some mechanism of decomposition of fatty acid peroxides by a glutathione peroxidase. They suggested that this enzymatic process could be coupled to the functioning of the flavins. As shown by DE VOS [8] the flavo-proteins are absent in the foetal liver of rats till 5 days before birth, and increase very rapidly afterwards. The process of direct oxidation of the reduced flavins by O_2 , provides H_2O_2 ; we could assume that an increased production of H_2O_2 could play the role of an inducer of the activity of GPX.

A steep rise of the activity of GPX was found during puberty in the maturing female. KARLSON [9] described inducing effects of steroid hormones on enzymatic activities. ROUX and TORDET-CARL-DROIT [10] have found in developing rat liver a difference in the activity of G_6PDH linked to sex. These authors were able to show by means of castration and hormone injection (testosterone and oestradiol benzoate) that oestradiol increased enzymatic activity, whereas testosterone played the role of a negative inducer. FEIGELSON [11] studying the hepatic histidase in rat liver during development found a similar pattern with a sexual dichotomy in the adult. The developmental and hormonally induced rise of activity were both inhibited by L-ethionine and actinomycin D.

The following questions were now raised for GPX. Could the high levels of activity, in the case of female, be depressed by ovariectomy or by testosterone injection or restored by oestradiol after

castration? Some experiments are now carried out in our laboratory upon this subject. The break off of the maternal oestrogen supply after birth, could explain in part the decrease in activity observed at 48 h by newborn rats.

At the same time investigation with RNA and protein synthesis inhibitors are undertaken to elucidate whether the rise in catalytic activity of GPX could be related to an increase in the synthesis of new enzyme molecules.

The striking difference in activity found between hypotrophic and normal rats at the same stage of development will be discussed in a further publication summarizing the over-all pattern of GPX activity of hypotrophic rats from birth to four months of age.

Summary

The activity of GPX in the soluble fraction of rat liver is low at birth and increases 7-fold in male and 15-fold in female animals during postnatal development. A transient decrease of activity is found at 48 h. The difference in activity, which is sex-dependent, is first observed during puberty. At four months of age the average activity found in the female rat is 50% higher than in the male. An enzyme induction by sexual hormones is discussed. During the first days of life GPX activity is found lower in hypotrophic rats as compared to the normal controls.

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